SALT ANTAGONISM IN GELATINE

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In studying the effects of electrolytes on living organisms Loeb[1] has distinguished two groups of salt antagonisms. To the first belongs the antagonism between NaCl and $CaCl_2$. Here the antagonism is between a monovalent salt and a salt with a strong (bivalent or tri-valent) cation. The antagonistic effect increases with the valence of the cation. To the second group belongs the inhibition of the toxic action of KCl on the eggs of *Fundulus* by means of sodium salts, the sulphate and citrate being the most effective. In this second group the antagonism is between a monovalent salt and a salt with a strong (bivalent or trivalent) anion, the effect increasing with the valence of the anion.

A recent explanation of the first kind of antagonism has been presented by Clowes,[2] who assumes that NaCl has a more strongly adsorbed anion and is therefore able to antagonize more strongly adsorbed cations (like Ca). His general conclusion is that electrolytes with a more strongly adsorbed anion are able to antagonize electrolytes with a more strongly adsorbed cation. No explanation is given of antagonism between monovalent salts and salts with strong anions (like Na_2SO_4).

From experiments on gelatine the writer has formulated a hypo-thesis by which both groups of antagonism may be explained. Accord-ing to this hypothesis (in many respects similar to that of Clowes) electrolytes with a more strongly adsorbed cation should antagonize electrolytes with a more strongly adsorbed anion, *and in the case of salts like NaCl, the effect of the Na ion should predominate in the presence of strong anions and the effect of the Cl ion should predominate in the presence of strong cations.*

In agreement with this hypothesis it is found possible to duplicate experimentally both groups of antagonism, as defined by Loeb, i.e., NaCl is found to antagonize both anions and cations. This is not only in agreement with biological results, but it also correlates the well known fact that NaCl tends to maintain the neutrality of protein sols to which either acid or alkali have been added,[3] and to decrease the viscosity of both alike.[4]

The results of the gelatine experiments in so far as they concern antagonism are represented more completely in figure 1 in diagrammatic

form. Only a few typical electrolytes are included in the diagram, but the general principles which they exemplify are applicable to all cases thus far studied.

According to this scheme, electrolytes are found to fall into three groups.

Group I includes salts with monovalent cations and monovalent anions, the effect of the former predominating in the presence of Group II, the effect of the latter in the presence of Group III.

Group II includes alkalies and salts with bi- and trivalent anions, the effect of the anion predominating.

Group III includes acids, and salts with bi- and tri-valent cations, the effect of the cations predominating.

Members of any one group are additive to each other but antagonistic to members of either of the other two groups. In general the antagonism between Groups II and III is greater than between Groups I and II
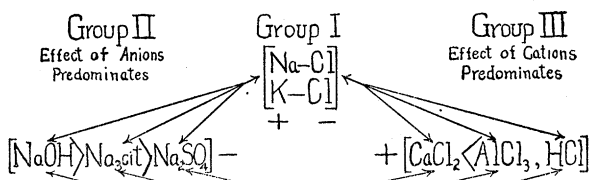

FIG. 1

Aditive combinations are bracketted together. Antagonistic combinations are connected by arrows. In general, the length of the arrows indicates the amount of antagonism expected.

or between Groups I and III. The valence of the ion (except H and OH), while not the only factor, seems of chief importance. Acids and alkalies are in general more effective than neutral salts.

An exception to this rule is found in the case of $MgCl_2$ which is found to antagonize both NaCl and $CaCl_2$. This exception, however, is in agreement with biological results.

As a criterion of antagonism, the precipitation of gelatine by 95% alcohol was used. It is found that *less alcohol is required for the precipitation of the gelatine in the presence of two antagonistic salts than when either is present alone.*

Let us for convenience designate the antagonistic salts as A and B. We find that as more and more of salt A is added to gelatine, more and more alcohol is required to produce an opaque precipitate, until finally a maximum is reached. This maximum, according to Pauli's theory,[5] corresponds to the greatest ionization of the protein and the greatest viscosity. At this point the hydration of the protein is greatest and

offers greatest resistance to the dehydration effect of the alcohol, by which precipitation is ultimately accomplished.

If now salt A be gradually replaced by an antagonistic salt B, less and less alcohol is required for precipitation until a minimum is reached. This minimum is at the isoelectric point, corresponding to greatest aggregation. *At this point, if the concentration of the salts be sufficiently high the gelatine is precipitated without alcohol.* If all of salt A be replaced by salt B, the amount of alcohol needed for precipitation again rises to the point characteristic of that salt in the concentration used. Thus an antagonism curve can be plotted, such as that shown in figure 2 for $CaCl_2$ and sodium citrate and in figure 3 for NaCl and $CaCl_2$. The probable additive effect in each figure is represented by the dotted base line. Such lines at least have resulted from similarly arranged experiments with non-antagonistic salts such as NaCl vs. KSCN and $CaCl_2$ vs. $MnCl_2$.[6]

It is frequently more convenient in determining antagonism to keep the concentration of one salt constant and gradually increase that of the other. If the two salts are *additive* to each other, the alcohol needed for precipitation is thereby *increased*. Thus when $CaCl_2$ is added to gelatine containing HCl, or when Na_2SO_4 is added to gelatine containing NaOH, the amount of alcohol needed for precipitation is increased.[7] If two *antagonistic* salts are used, on the other hand, there is a *decrease* in the amount of alcohol needed for precipitation until a minimum is reached. This is again an isoelectric point and here the gelatine may be precipitated without alcohol if the salt concentration be sufficiently high. If still more of the salt be added, there is an increase of the amount of alcohol needed for precipitation and a decrease of precipitability by salts in the absence of alcohol. Such results are observed when NaCl, for example, is added to gelatine containing HCl, NaOH, $AlCl_3$, $CaCl_2$, Na_2SO_4 or Na_3-citrate.

The molecular proportions of salts at the minimum, i.e. at the isoelectric point or point of greatest precipitability, appears to be characteristic of the particular salts used. Thus for NaCl and $CaCl_2$, it is about 100 to 20; for NaCl and $AlCl_3$, about 100 to 1.5.

Mixtures of NaCl and $AlCl_3$ in the above proportions[8] gives maximum precipitation in gelatine, milk, and olive oil emulsions.[9] Gelatine containing NaCl 0.5M shows maximum precipitation in presence of $AlCl_3$ 0.0078M and $Ce(NO)_3$ 0.00625M. The molecular proportions at these two points are 100 to 1.5 and 100 to 1.2. Mines[10] has observed a maximum precipitation of blood corpuscles in NaCl plus $CeCl_3$, the molecular proportions being between 100 to 3.3 and 100 to 0.03.
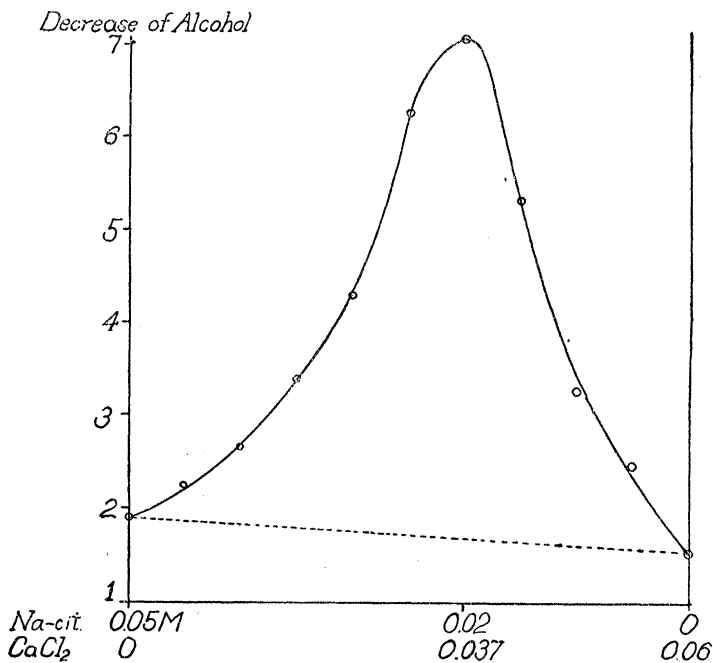
FIG. 2

Antagonism between $CaCl_2$ and Na-citrate in gelatine. Decrease of alcohol required for precipitation is plotted as ordinates (as 12 minus the number of cc. of 95% alcohol added to 5 cc. of the gelatine-salt mixtures in order to produce an opaque precipitate). As abscissae are plotted the concentrations of NaCl and $CaCl_2$ simultaneously present in the gelatine before titrating. The dotted line represents the additive effect. A pure grade of commerical gelatine was used in 3% solution. Each point represents the mean of two titrations. Each titration is accurate to 0.2 cc. The gelatine was always allowed to stand at least 15 hours in an incubator at 26°C. in order to reach equilibrium.
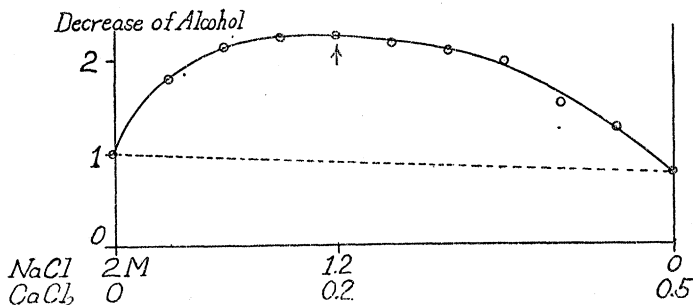

FIG. 3

Antagonism between NaCl and $CaCl_2$ in gelatine (for explanation see figure 2). **At the** maximum the molecular proportion of NaCl to $CaCl_2$ is 100 to 17.

In pure NaCl there was no precipitation and the corpuscles wandered to the anode. In excess of cerium there was no precipitation and the corpuscles wandered to the cathode. These results become intelligible if the point of maximum precipitation is an isoelectric point due to the predominating effect of the Cl ion (of NaCl) in the presence of the trivalent cerium cation.

From these experiments it may be reasoned that antagonistic salt solutions produce a precipitate or a state of aggregation of an iso-electric nature in protoplasm. Physicochemically, therefore, a physio-logically balanced solution would be merely a mixture producing high precipitability. By varying the amount of the precipitate changes in the permeability of the protoplasm might be brought about.

In connection with the hypothesis developed here attention may be called to the suggestion of Loeb[11] that antagonism between salts and acids furnishes an excellent parallel to the results of Procter on gelatin and indicates that the protoplasmic substances which are responsible for antagonism are protein in nature.

Summary.—1. The experiments on gelatine support the hypothesis that anions antagonize cations in their effects upon organisms.

2. The hypothesis here developed resembles that of Clowes except that it requires that NaCl should antagonize any electrolyte which has either a strong anion or a strong cation.

3. The point of maximum antagonism is an isoelectric point at which the amount of alcohol needed for precipitation is at a minimum, and the aggregation or amount of precipitation is at a maximum.

[1] Loeb, These PROCEEDINGS, **1**, 473 (1915).

[2] Clowes, *J. Phys. Chem.*, **20**, 407 (1916) and *Science*, **43**, 750.

[3] Chick and Martin, *Koll.-Chem. Beihefte*, **5**, 49 (1915).

[4] Pauli, *Biochem. Zs.*, **24**, 239 (1910).

[5] Pauli, *Kolloid Zs.*, **12**, 222 (1913); **7**, 241 (1910).

[4] Pauli u. Wagner, *Biochem. Zs.*, **27**, 296 (1910).

[6] For "additive effect" see Osterhout, *Bot. Gaz.*, **58**, 178 (1914).

[7] This is true at least in low concentrations. In higher concentrations, the results are complicated by other factors and have not been fully determined.

[8] These proportions would be expected to vary with the medium used and the conditions of the experiment. Clowes (l.c.) finds that the critical point for NaCl and $CaCl_2$ in oil-water emulsions varies between 500 to 1 and 10 to 1.

[9] Clowes has shown that in critical mixtures of NaCl and $CaCl_2$, the phases of a water-oil emulsion separate out.

[10] Mines, G. R., *Koll.-Chem. Beihefte* **3**, 191 (1912).

[11] *Science*, **34**, 653 (1911); *Biochem. Zs.* **33**, 489 (1911) and **47**, 127 (1912).